

SEQUENCE LISTING

<110>KAO CORPORATION

<120>New mutant alpha-amylase

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<150>JP P1999-163569

<151>1999-06-10

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<213>Bacillus sp. KSM-K38

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Asn Asp Gly Gln His Trp Asn Arg Leu His Asp Asp Ala Ala Ala Leu

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Ser Asp Ala Gly Ile Thr Ala Ile Trp Ile Pro Pro Ala Tyr Lys Gly

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Asn Ser Gln Ala Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr Asp Leu

50	55	60
Gly Glu Phe Asn Gln Lys	Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys	
65	70	75
Ala Gln Leu Glu Arg Ala Ile Gly Ser Leu Lys Ser Asn Asp Ile Asn		80
	85	90
Val Tyr Gly Asp Val Val Met Asn His Lys Met Gly Ala Asp Phe Thr		95
100	105	110
Glu Ala Val Gln Ala Val Gln Val Asn Pro Thr Asn Arg Trp Gln Asp		
115	120	125
Ile Ser Gly Ala Tyr Thr Ile Asp Ala Trp Thr Gly Phe Asp Phe Ser		
130	135	140
Gly Arg Asn Asn Ala Tyr Ser Asp Phe Lys Trp Arg Trp Phe His Phe		
145	150	155
Asn Gly Val Asp Trp Asp Gln Arg Tyr Gln Glu Asn His Ile Phe Arg		160
	165	170
Phe Ala Asn Thr Asn Trp Asn Trp Arg Val Asp Glu Glu Asn Gly Asn		175
180	185	190
Tyr Asp Tyr Leu Leu Gly Ser Asn Ile Asp Phe Ser His Pro Glu Val		
195	200	205
Gln Asp Glu Leu Lys Asp Trp Gly Ser Trp Phe Thr Asp Glu Leu Asp		
210	215	220
Leu Asp Gly Tyr Arg Leu Asp Ala Ile Lys His Ile Pro Phe Trp Tyr		
225	230	235
Thr Ser Asp Trp Val Arg His Gln Arg Asn Glu Ala Asp Gln Asp Leu		240
	245	250
Phe Val Val Gly Glu Tyr Trp Lys Asp Asp Val Gly Ala Leu Glu Phe		255

260	265	270
Tyr Leu Asp Glu Met Asn Trp Glu Met Ser Leu Phe Asp Val Pro Leu		
275	280	285
Asn Tyr Asn Phe Tyr Arg Ala Ser Gln Gln Gly Gly Ser Tyr Asp Met		
290	295	300
Arg Asn Ile Leu Arg Gly Ser Leu Val Glu Ala His Pro Met His Ala		
305	310	315
Val Thr Phe Val Asp Asn His Asp Thr Gln Pro Gly Glu Ser Leu Glu		
325	330	335
Ser Trp Val Ala Asp Trp Phe Lys Pro Leu Ala Tyr Ala Thr Ile Leu		
340	345	350
Thr Arg Glu Gly Gly Tyr Pro Asn Val Phe Tyr Gly Asp Tyr Tyr Gly		
355	360	365
Ile Pro Asn Asp Asn Ile Ser Ala Lys Lys Asp Met Ile Asp Glu Leu		
370	375	380
Leu Asp Ala Arg Gln Asn Tyr Ala Tyr Gly Thr Gln His Asp Tyr Phe		
385	390	395
Asp His Trp Asp Val Val Gly Trp Thr Arg Glu Gly Ser Ser Ser Arg		
405	410	415
Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asn Gly Pro Gly Gly Ser		
420	425	430
Lys Trp Met Tyr Val Gly Arg Gln Asn Ala Gly Gln Thr Trp Thr Asp		
435	440	445
Leu Thr Gly Asn Asn Gly Ala Ser Val Thr Ile Asn Gly Asp Gly Trp		
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<213>Bacillus sp. KSM-AP1378

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30

Asn Leu Lys Ser Lys Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Trp

35

40

45

Lys Gly Thr Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr

50

55

60

Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly

65

70

75

80

Thr Arg Ser Gln Leu Gln Gly Ala Val Thr Ser Leu Lys Asn Asn Gly

85

90

95

Ile Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp

100

105

110

Gly Thr Glu Met Val Asn Ala Val Glu Val Asn Arg Ser Asn Arg Asn

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Gln Glu Ile Ser Gly Glu Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp

130

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140

Phe	Pro	Gly	Arg	Gly	Asn	Thr	His	Ser	Asn	Phe	Lys	Trp	Arg	Trp	Tyr
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His	Phe	Asp	Gly	Thr	Asp	Trp	Asp	Gln	Ser	Arg	Gln	Leu	Gln	Asn	Lys
				165					170					175	
Ile	Tyr	Lys	Phe	Arg	Gly	Thr	Gly	Lys	Ala	Trp	Asp	Trp	Glu	Val	Asp
				180					185					190	
Ile	Glu	Asn	Gly	Asn	Tyr	Asp	Tyr	Leu	Met	Tyr	Ala	Asp	Ile	Asp	Met
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Asp	His	Pro	Glu	Val	Ile	Asn	Glu	Leu	Arg	Asn	Trp	Gly	Val	Trp	Tyr
				210				215				220			
Thr	Asn	Thr	Leu	Asn	Leu	Asp	Gly	Phe	Arg	Ile	Asp	Ala	Val	Lys	His
225					230					235					240
Ile	Lys	Tyr	Ser	Tyr	Thr	Arg	Asp	Trp	Leu	Thr	His	Val	Arg	Asn	Thr
					245				250					255	
Thr	Gly	Lys	Pro	Met	Phe	Ala	Val	Ala	Glu	Phe	Trp	Lys	Asn	Asp	Leu
				260					265					270	
Ala	Ala	Ile	Glu	Asn	Tyr	Leu	Asn	Lys	Thr	Ser	Trp	Asn	His	Ser	Val
				275					280					285	
Phe	Asp	Val	Pro	Leu	His	Tyr	Asn	Leu	Tyr	Asn	Ala	Ser	Asn	Ser	Gly
				290				295				300			
Gly	Tyr	Phe	Asp	Met	Arg	Asn	Ile	Leu	Asn	Gly	Ser	Val	Val	Gln	Lys
305					310					315					320
His	Pro	Ile	His	Ala	Val	Thr	Phe	Val	Asp	Asn	His	Asp	Ser	Gln	Pro
				325						330				335	
Gly	Glu	Ala	Leu	Glu	Ser	Phe	Val	Gln	Ser	Trp	Phe	Lys	Pro	Leu	Ala
				340					345					350	

Tyr Ala Leu Ile Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr
 355 360 365
 Gly Asp Tyr Tyr Gly Ile Pro Thr His Gly Val Pro Ser Met Lys Ser
 370 375 380
 Lys Ile Asp Pro Leu Leu Gln Ala Arg Gln Thr Tyr Ala Tyr Gly Thr
 385 390 395 400
 Gln His Asp Tyr Phe Asp His His Asp Ile Ile Gly Trp Thr Arg Glu
 405 410 415
 Gly Asp Ser Ser His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp
 420 425 430
 Gly Pro Gly Gly Asn Lys Trp Met Tyr Val Gly Lys His Lys Ala Gly
 435 440 445
 Gln Val Trp Arg Asp Ile Thr Gly Asn Arg Ser Gly Thr Val Thr Ile
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<213>Bacillus sp. KSM-K38

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ttaatgatata tgtaagcggt atcattaaaa ggaggatatt g atg aga aga tgg gta 176
gta gca atg ttg gca gtg tta ttt tta ttt cct tgc gta gta gtt gca 224
gat gga ttg aac ggt acg atg atg cag tat tat gag tgg cat ttg gaa 272
Asp Gly Leu Asn Gly Thr Met Met Gln Tyr Tyr Glu Trp His Leu Glu
1          5          10          15
aac gac ggg cag cat tgg aat cgg ttg cac gat gat gcc gca gct ttg 320
Asn Asp Gly Gln His Trp Asn Arg Leu His Asp Asp Ala Ala Ala Leu
20          25          30
agt gat gct ggt att aca gct att tgg att ccg cca gcc tac aaa ggt 368
Ser Asp Ala Gly Ile Thr Ala Ile Trp Ile Pro Pro Ala Tyr Lys Gly
35          40          45
aat agt cag gcg gat gtt ggg tac ggt gca tac gat ctt tat gat tta 416
Asn Ser Gln Ala Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr Asp Leu
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Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys			
65	70	75	80
gca cag ctt gaa cga gct att ggg tcc ctt aaa tct aat gat atc aat	512		
Ala Gln Leu Glu Arg Ala Ile Gly Ser Leu Lys Ser Asn Asp Ile Asn			
85	90	95	
gta tac gga gat gtc gtg atg aat cat aaa atg gga gct gat ttt acg	560		
Val Tyr Gly Asp Val Val Met Asn His Lys Met Gly Ala Asp Phe Thr			
100	105	110	
gag gca gtg caa gct gtt caa gta aat cca acg aat cgt tgg cag gat	608		
Glu Ala Val Gln Ala Val Gln Val Asn Pro Thr Asn Arg Trp Gln Asp			
115	120	125	
att tca ggt gcc tac acg att gat gcg tgg acg ggt ttc gac ttt tca	656		
Ile Ser Gly Ala Tyr Thr Ile Asp Ala Trp Thr Gly Phe Asp Phe Ser			
130	135	140	
ggg cgt aac aac gcc tat tca gat ttt aag tgg aga tgg ttc cat ttt	704		
Gly Arg Asn Asn Ala Tyr Ser Asp Phe Lys Trp Arg Trp Phe His Phe			
145	150	155	160
aat ggt gtt gac tgg gat cag cgc tat caa gaa aat cat att ttc cgc	752		
Asn Gly Val Asp Trp Asp Gln Arg Tyr Gln Glu Asn His Ile Phe Arg			
165	170	175	
ttt gca aat acg aac tgg aac tgg cga gtg gat gaa gag aac ggt aat	800		
Phe Ala Asn Thr Asn Trp Asn Trp Arg Val Asp Glu Glu Asn Gly Asn			
180	185	190	
tat gat tac ctg tta gga tcg aat atc gac ttt agt cat cca gaa gta	848		

Tyr Asp Tyr Leu Leu Gly Ser Asn Ile Asp Phe Ser His Pro Glu Val	
195 200 205	
caa gat gag ttg aag gat tgg ggt agc tgg ttt acc gat gag tta gat	896
Gln Asp Glu Leu Lys Asp Trp Gly Ser Trp Phe Thr Asp Glu Leu Asp	
210 215 220	
ttg gat ggt tat cgt tta gat gct att aaa cat att cca ttc tgg tat	944
Leu Asp Gly Tyr Arg Leu Asp Ala Ile Lys His Ile Pro Phe Trp Tyr	
225 230 235 240	
aca tct gat tgg gtt cgg cat cag cgc aac gaa gca gat caa gat tta	992
Thr Ser Asp Trp Val Arg His Gln Arg Asn Glu Ala Asp Gln Asp Leu	
245 250 255	
ttt gtc gta ggg gaa tat tgg aag gat gac gta ggt gct ctc gaa ttt	1040
Phe Val Val Gly Glu Tyr Trp Lys Asp Asp Val Gly Ala Leu Glu Phe	
260 265 270	
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Tyr Leu Asp Glu Met Asn Trp Glu Met Ser Leu Phe Asp Val Pro Leu	
275 280 285	
aat tat aat ttt tac cgg gct tca caa caa ggt gga agc tat gat atg	1136
Asn Tyr Asn Phe Tyr Arg Ala Ser Gln Gln Gly Gly Ser Tyr Asp Met	
290 295 300	
cgt aat att tta cga gga tct tta gta gaa gcg cat ccg atg cat gca	1184
Arg Asn Ile Leu Arg Gly Ser Leu Val Glu Ala His Pro Met His Ala	
305 310 315 320	
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Val Thr Phe Val Asp Asn His Asp Thr Gln Pro Gly Glu Ser Leu Glu	
325 330 335	

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Ser Trp Val Ala Asp Trp Phe Lys Pro Leu Ala Tyr Ala Thr Ile Leu	
340 345 350	
acg cgt gaa ggt ggt tat cca aat gta ttt tac ggt gat tac tat ggg	1328
Thr Arg Glu Gly Gly Tyr Pro Asn Val Phe Tyr Gly Asp Tyr Tyr Gly	
355 360 365	
att cct aac gat aac att tca gct aaa aaa gat atg att gat gag ctg	1376
Ile Pro Asn Asp Asn Ile Ser Ala Lys Lys Asp Met Ile Asp Glu Leu	
370 375 380	
ctt gat gca cgt caa aat tac gca tat ggc acg cag cat gac tat ttt	1424
Leu Asp Ala Arg Gln Asn Tyr Ala Tyr Gly Thr Gln His Asp Tyr Phe	
385 390 395 400	
gat cat tgg gat gtt gta gga tgg act agg gaa gga tct tcc tcc aga	1472
Asp His Trp Asp Val Val Gly Trp Thr Arg Glu Gly Ser Ser Ser Arg	
405 410 415	
cct aat tca ggc ctt gcg act att atg tcg aat gga cct ggt ggt tcc	1520
Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asn Gly Pro Gly Gly Ser	
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aag tgg atg tat gta gga cgt cag aat gca gga caa aca tgg aca gat	1568
Lys Trp Met Tyr Val Gly Arg Gln Asn Ala Gly Gln Thr Trp Thr Asp	
435 440 445	
tta act ggt aat aac gga gcg tcc gtt aca att aat ggc gat gga tgg	1616
Leu Thr Gly Asn Asn Gly Ala Ser Val Thr Ile Asn Gly Asp Gly Trp	
450 455 460	
ggc gaa ttc ttt acg aat gga gga tct gta tcc gtg tac gtg aac caa	1664

Gly Glu Phe Phe Thr Asn Gly Gly Ser Val Ser Val Tyr Val Asn Gln
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<212>PRT

<213>Bacillus sp. KSM-K36

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 35 40 45
 Asn Ser Gln Ala Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr Asp Leu
 50 55 60
 Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys
 65 70 75 80
 Ala Gln Leu Glu Arg Ala Ile Gly Ser Leu Lys Ser Asn Asp Ile Asn
 85 90 95
 Val Tyr Gly Asp Val Val Met Asn His Lys Leu Gly Ala Asp Phe Thr
 100 105 110
 Glu Ala Val Gln Ala Val Gln Val Asn Pro Ser Asn Arg Trp Gln Asp

115	120	125
Ile Ser Gly Val Tyr Thr	Ile Asp Ala Trp Thr	Gly Phe Asp Phe Pro
130	135	140
Gly Arg Asn Asn Ala Tyr	Ser Asp Phe Lys Trp Arg	Trp Phe His Phe
145	150	155
Asn Gly Val Asp Trp Asp	Gln Arg Tyr Gln Glu Asn	His Leu Phe Arg
165	170	175
Phe Ala Asn Thr Asn Trp	Asn Trp Arg Val Asp	Glu Glu Asn Gly Asn
180	185	190
Tyr Asp Tyr Leu Leu Gly	Ser Asn Ile Asp Phe Ser	His Pro Glu Val
195	200	205
Gln Glu Glu Leu Lys Asp	Trp Gly Ser Trp Phe Thr	Asp Glu Leu Asp
210	215	220
Leu Asp Gly Tyr Arg Leu	Asp Ala Ile Lys His Ile	Pro Phe Trp Tyr
225	230	235
Thr Ser Asp Trp Val Arg	His Gln Arg Ser Glu Ala	Asp Gln Asp Leu
245	250	255
Phe Val Val Gly Glu Tyr	Trp Lys Asp Asp Val Gly	Ala Leu Glu Phe
260	265	270
Tyr Leu Asp Glu Met Asn	Trp Glu Met Ser Leu Phe	Asp Val Pro Leu
275	280	285
Asn Tyr Asn Phe Tyr Arg	Ala Ser Lys Gln Gly Gly	Ser Tyr Asp Met
290	295	300
Arg Asn Ile Leu Arg Gly	Ser Leu Val Glu Ala His	Pro Ile His Ala
305	310	315
Val Thr Phe Val Asp Asn	His Asp Thr Gln Pro Gly	Glu Ser Leu Glu

	325	330	335
Ser Trp Val Ala Asp Trp Phe Lys Pro Leu Ala Tyr Ala Thr Ile Leu			
	340	345	350
Thr Arg Glu Gly Gly Tyr Pro Asn Val Phe Tyr Gly Asp Tyr Tyr Gly			
	355	360	365
Ile Pro Asn Asp Asn Ile Ser Ala Lys Lys Asp Met Ile Asp Glu Leu			
	370	375	380
Leu Asp Ala Arg Gln Asn Tyr Ala Tyr Gly Thr Gln His Asp Tyr Phe			
385	390	395	400
Asp His Trp Asp Ile Val Gly Trp Thr Arg Glu Gly Thr Ser Ser Arg			
	405	410	415
Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asn Gly Pro Gly Gly Ser			
	420	425	430
Lys Trp Met Tyr Val Gly Gln Gln His Ala Gly Gln Thr Trp Thr Asp			
	435	440	445
Leu Thr Gly Asn His Ala Ala Ser Val Thr Ile Asn Gly Asp Gly Trp			
	450	455	460
Gly Glu Phe Phe Thr Asn Gly Gly Ser Val Ser Val Tyr Val Asn Gln			
465	470	475	480

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gat ggc ttg aat gga acg atg atg cag tat tat gag tgg cat cta gag      150
Asp Gly Leu Asn Gly Thr Met Met Gln Tyr Tyr Glu Trp His Leu Glu
  1             5             10             15
aat gat ggg caa cac tgg aat cgg ttg cat gat gat gcc gaa gct tta      198
Asn Asp Gly Gln His Trp Asn Arg Leu His Asp Asp Ala Glu Ala Leu
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agt aat gcg ggt att aca gct att tgg ata ccc cca gcc tac aaa gga      246
Ser Asn Ala Gly Ile Thr Ala Ile Trp Ile Pro Pro Ala Tyr Lys Gly
      35             40             45
aat agt cag gct gat gtt ggg tat ggt gca tac gac ctt tat gat tta      294
Asn Ser Gln Ala Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr Asp Leu
      50             55             60
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Ala Gln Leu Glu Arg Ala Ile Gly Ser Leu Lys Ser Asn Asp Ile Asn	
85 90 95	
gtt tat ggg gat gtc gta atg aat cat aaa tta gga gct gat ttc acg	438
Val Tyr Gly Asp Val Val Met Asn His Lys Leu Gly Ala Asp Phe Thr	
100 105 110	
gag gca gtg caa gct gtt caa gta aat cct tcg aac cgt tgg cag gat	486
Glu Ala Val Gln Ala Val Gln Val Asn Pro Ser Asn Arg Trp Gln Asp	
115 120 125	
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Ile Ser Gly Val Tyr Thr Ile Asp Ala Trp Thr Gly Phe Asp Phe Pro	
130 135 140	
ggg cgc aac aat gcc tat tcc gat ttt aaa tgg aga tgg ttc cat ttt	582
Gly Arg Asn Asn Ala Tyr Ser Asp Phe Lys Trp Arg Trp Phe His Phe	
145 150 155 160	
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Asn Gly Val Asp Trp Asp Gln Arg Tyr Gln Glu Asn His Leu Phe Arg	
165 170 175	
ttt gca aat acg aac tgg aac tgg cga gtg gat gaa gag aat ggt aat	678
Phe Ala Asn Thr Asn Trp Asn Trp Arg Val Asp Glu Glu Asn Gly Asn	
180 185 190	
tat gac tat tta tta gga tcg aac att gac ttt agc cac cca gag gtt	726
Tyr Asp Tyr Leu Leu Gly Ser Asn Ile Asp Phe Ser His Pro Glu Val	

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Gln Glu Glu Leu Lys Asp Trp Gly Ser Trp Phe Thr Asp Glu Leu Asp			
210	215	220	
tta gat ggg tat cga ttg gat gct att aag cat att cca ttc tgg tat			822
Leu Asp Gly Tyr Arg Leu Asp Ala Ile Lys His Ile Pro Phe Trp Tyr			
225	230	235	240
acg tca gat tgg gtt agg cat cag cga agt gaa gca gac caa gat tta			870
Thr Ser Asp Trp Val Arg His Gln Arg Ser Glu Ala Asp Gln Asp Leu			
245	250	255	
ttt gtc gta ggg gag tat tgg aag gat gac gta ggt gct ctc gaa ttt			918
Phe Val Val Gly Glu Tyr Trp Lys Asp Asp Val Gly Ala Leu Glu Phe			
260	265	270	
tat tta gat gaa atg aat tgg gag atg tct cta ttc gat gtt ccg ctc			966
Tyr Leu Asp Glu Met Asn Trp Glu Met Ser Leu Phe Asp Val Pro Leu			
275	280	285	
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Arg Asn Ile Leu Arg Gly Ser Leu Val Glu Ala His Pro Ile His Ala			
305	310	315	320
gtt acg ttt gtt gat aat cat gat act cag cca gga gag tca tta gaa			1110
Val Thr Phe Val Asp Asn His Asp Thr Gln Pro Gly Glu Ser Leu Glu			
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Thr Arg Glu Gly Gly Tyr Pro Asn Val Phe Tyr Gly Asp Tyr Tyr Gly	
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Ile Pro Asn Asp Asn Ile Ser Ala Lys Lys Asp Met Ile Asp Glu Leu	
370 375 380	
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Leu Asp Ala Arg Gln Asn Tyr Ala Tyr Gly Thr Gln His Asp Tyr Phe	
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Asp His Trp Asp Ile Val Gly Trp Thr Arg Glu Gly Thr Ser Ser Arg	
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cct aat tcg ggt ctt gct act att atg tcc aat ggt cct gga gga tca	1398
Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asn Gly Pro Gly Gly Ser	
420 425 430	
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Lys Trp Met Tyr Val Gly Gln Gln His Ala Gly Gln Thr Trp Thr Asp	
435 440 445	
tta act ggc aat cac gcg gcg tcg gtt acg att aat ggt gat ggc tgg	1494
Leu Thr Gly Asn His Ala Ala Ser Val Thr Ile Asn Gly Asp Gly Trp	
450 455 460	
ggc gaa ttc ttt aca aat gga gga tct gta tcc gtg tat gtg aac caa	1542
Gly Glu Phe Phe Thr Asn Gly Gly Ser Val Ser Val Tyr Val Asn Gln	
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<213>Artificial Sequence

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